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Anticariogenic Activity of Copper Nanoparticles Synthesized Using Blue Tea

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Nanotechnology is rapidly growing in various fields of science like medicinal, agricultural and physical and material sciences. Copper nanoparticles are particularly attractive because of copper's high natural abundance and low cost and the practical and straightforward multiple ways of preparing copper based nanomaterials.
Aim: To assess the anticariogenic activity of copper nanoparticles synthesized using blue tea.
Materials and Methods: The blue tea powder was acquired. An aqueous extract was prepared and mixed with copper sulphate for copper nanoparticles formation and centrifuged for 10 minutes. The extract was then placed in the well cultured agar plates against *C. albicans, S. mutans, S. aureus* and *E. faecalis* and incubated for 24 hours. The zones of inhibition were then calculated.
Results: Against *S. aureus*, 25µl showed 10 mm of zone of inhibition, 50µl showed 10 mm of zone of inhibition and 100µl showed 12 mm of zone of inhibition. 21 mm of zone of inhibition were noted against the antibiotic. Against *C. albicans*, 25 µl showed 25 mm of zone of inhibition, 50 µl showed 26 mm of zone of inhibition and 100 µl showed 30 mm of zone of inhibition. 12 mm of zone of

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inhibition were noted against the antibiotic. Against *S. mutans*, 25 µl showed 10 mm of zone of inhibition, 50 µl showed 10 mm of zone of inhibition and 100 µl showed 25 mm of zone of inhibition. 21 mm of zone of inhibition were noted against the antibiotic. Against *E. faecalis*, 25 µl showed 10 mm of zone of inhibition, 50 µl showed 13 mm of zone of inhibition and 100 µl showed 14 mm of zone of inhibition. 37 mm of zone of inhibition were noted against the antibiotic.

Conclusion: The blue tea mediated copper nanoparticles showed anticariogenic activity against *S. mutans, C. albicans, E. faecalis* and *S. aureus* and therefore can be used for clinical application.

Keywords: Anticariogenic; copper nanoparticles; Streptococcus mutans; blue tea; innovative; green synthesis.

1. INTRODUCTION

Nanotechnology is a significant field of present day research managing the plan, blend, and control of particles. Among different metals, copper nanoparticles are of extraordinary interest because of its minimal expense and simple accessibility with the property like other metal nanoparticles [1–7], [8]. Nanoparticles are the particles with size ranging from 1 nm to 100 nm providing solutions to environmental and technological challenges and applied in almost all the fields. Nanoparticles of copper and its compounds have been applied all the more regularly as impetuses due to their high surface-to-volume proportion and less cost contrasted with honorable metals. They are utilized as water gas shift impetus and gas detoxification impetuses [9], [10], [11].

The copper nanoparticles because of their unique physical and chemical properties, low cost preparation and less toxic nature have been a great interest to researchers and have become an active area in the academic field and most importantly in the field of nanoscience and technology [12]. Nanotechnology is mainly used to produce and process products eco-friendly and to minimize the use of hazardous environments containing antioxidant and antimicrobial properties are considered as a new trend of medicinal and therapeutic agents and even in the prevention of deterioration of food and pathogenic microorganisms. Moreover, copper is an inexpensive antimicrobial agent when compared to other agents like gold and silver. It has anticariogenic, antioxidant properties and longer shelf life when compared to other organic antimicrobial agents [13]. The presence of these unique physical, chemical and biological properties are due to their highly unusual crystal morphology and high surface area volume ratio [13,14].

Clitoria ternatea plant also referred to as the butterfly-blue pea, blue-pea, or Asian pigeon wings. The shape of the flowers of Clitoria ternatea was believed to resemble female human genitals, hence the name “Clitoria” from “Clitoris” [15]. , this bright-colored beverage has long been a part of the culture of Thailand, Vietnam, Bali, and Malaysia. Recent studies have shown blue tea to offer various health benefits, including immune-boosting properties, improved cognitive ability, and weight loss [16]. Blue tea has long been a part of Ayurveda and, as published in the Journal of Ethnopharmacology *“*it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizer and sedative agent [17]. There are no known side effects of blue tea, it is known to be extremely safe and super healthy to consume [18], [19], [20], [21]. However, over-consumption of Blue tea may cause nausea and diarrhea. Also, pregnant and lactating women are also advised to consult their doctor before drinking blue tea. Recent scientific endeavors suggest that blue tea may confer various antioxidant-associated health benefits including antimutagenic, anticarcinogenic, anti-inflammatory, and antiviral properties and antiatherosclerotic effects.

Our team has extensive knowledge and research experience that has translated into high quality publications [13,22–33],[34–38] [39], [40]. In this context, this study aims to assess the anticariogenic effect of copper nanoparticles synthesized using red tea.

2. MATERIALS AND METHODS

2.1 Preparation of the Extract

In a beaker, 1 g of freshly acquired blue tea powder was added to 100 ml of distilled water. It was mixed well and boiled for 5-10 minutes at 60-70°C (Fig. 1). The solution was then filtered using filter paper. The filtered extract was collected and stored.

Fig. 1. Blue tea powder mixed in distilled water

2.2 Synthesis of Nanoparticles

20mM of CuSO4 was added to the 20 ml of distilled water and kept in a magnetic stirrer for nanoparticle synthesis. The colour change was observed. Reading was noted every 2 hours. The solution of copper nanoparticles was centrifuged at 8000 rpm for 10 minutes. Then, the copper nanoparticles were collected and stored (Fig. 2).

2.3 Anticariogenic Activity

Agar well diffusion method was used to determine the anticariogenic activity of synthesised blue tea mediated copper nanoparticles. Different concentrations of copper nanoparticles were tested against *C. albicans, S. mutans, S. aureus* and *E. faecalis*. Different concentrations of copper nanoparticles (25 µl, 50 µl, 100 µl, Antibiotic) were incorporated into the prepared wells and the plates were incubated at 37°C for 24 hours to study its effect. Antibiotics (Amoxicillin) were used as positive control and the zones of inhibition were recorded. Two cups of butterfly-pea flower tea. The one on the right has had lime juice added, making it turn purple. One of the most distinctive characteristics of butterfly pea flower tea, and other drinks that use butterfly pea flower extract, is that it will change color when the pH balance changes. A deep blue tea will turn purple with the addition of lemon juice, turning a deeper shade of purple the more lemon juice is added. Mixed with fuchsia roselle hibiscus leaves the tea will turn a bright red color. A popular use of the tea is in cocktails where the showmanship of the cocktail making incorporates the instantaneous color change in front of the patron that ordered the drink.

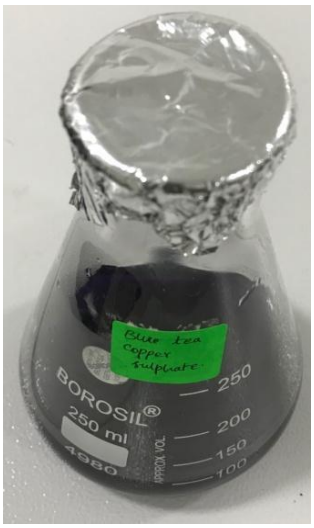

Fig. 2. Blue tea mediated copper nanoparticles

3. RESULTS

Zone of inhibition using different concentrations of red tea mediated copper nanoparticles shows anticariogenic activity against *S.aureus* (Fig. 3), *S.mutans* (Fig. 4), *C.albicans* (Fig. 5), *E.faecalis* (Fig. 6). Against *S. aureus*, 25µl showed 10 mm of zone of inhibition, 50µl showed 10 mm of zone of inhibition and 100µl showed 12 mm of zone of inhibition. 21 mm of zone of inhibition were noted against the antibiotic. Against *C. albicans*, 25 µl showed 25 mm of zone of inhibition, 50 µl showed 26 mm of zone of inhibition and 100 µl showed 30 mm of zone of inhibition. 12 mm of zone of inhibition were noted against the antibiotic. Against *S. mutans*, 25 µl showed 10 mm of zone of inhibition, 50 µl showed 10 mm of zone of inhibition and 100 µl showed 25 mm of zone of inhibition. 21 mm of zone of inhibition were noted against the antibiotic. Against *E. faecalis*, 25 µl showed 10 mm of zone of inhibition, 50 µl showed 13 mm of zone of inhibition and 100 µl showed 14 mm of zone of inhibition. 37 mm of zone of inhibition were noted against the antibiotic. (Table 1)

Zone of inhibition by disk-diffusion method shows anticariogenic activity in different concentrations of blue tea mediated copper nanoparticles (Fig. 7). Zones of inhibition obtained for different microorganisms at various concentrations of herbal extract were compared using ANOVA test. The results obtained for anticariogenic activity against *C. albicans* , *S. mutans* , *S. aureus* and *E. faecalis* was found to be statistically significant with the p value of <0.05 (Table 2 and Table 3).

Fig. 3. Zone of inhibition of blue tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. aureus* (25 µl, 50 µl, 100 µl, Antibiotic

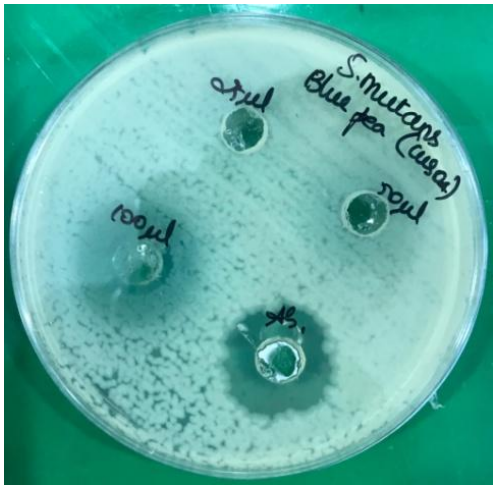

Fig. 4. Zone of inhibition of blue tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. mutans* (25 µl, 50 µl, 100 µl, Antibiotic)

Fig. 5. Zone of inhibition of blue tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *C. albicans* (25 µl, 50 µl, 100 µl, Antibiotic)

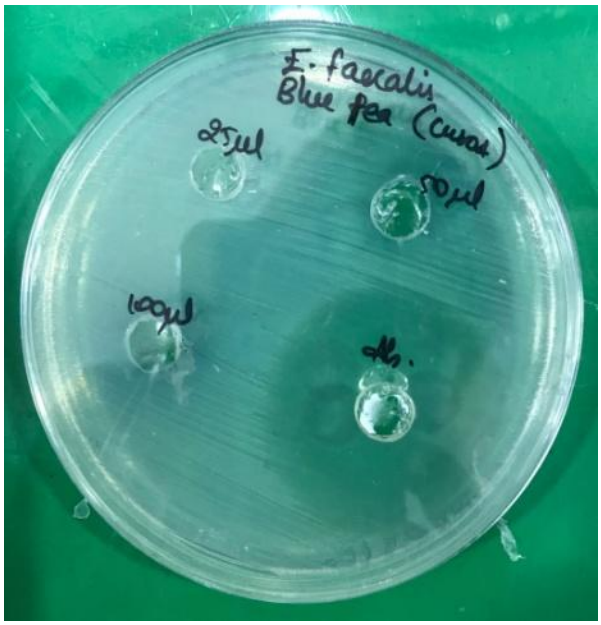

Fig. 6. Zone of inhibition of blue tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *E. faecalis* (25 µl, 50 µl, 100 µl, Antibiotic

Table 1. Zone of inhibition using different concentrations of blue tea mediated copper nanoparticles against *C. albicans, S. mutans, S. aureus and E. faecalis* (25 µl, 50 µl, 100 µl, Antibiotic

Concentration (micro litres)	*S.aureus*	*S.mutans*	*C. albicans*	*E.faecalis*
25µl	10	10	25	10
50µl	10	10	26	13
100µl	12	25	30	14
Antibiotic	21	21	12	37

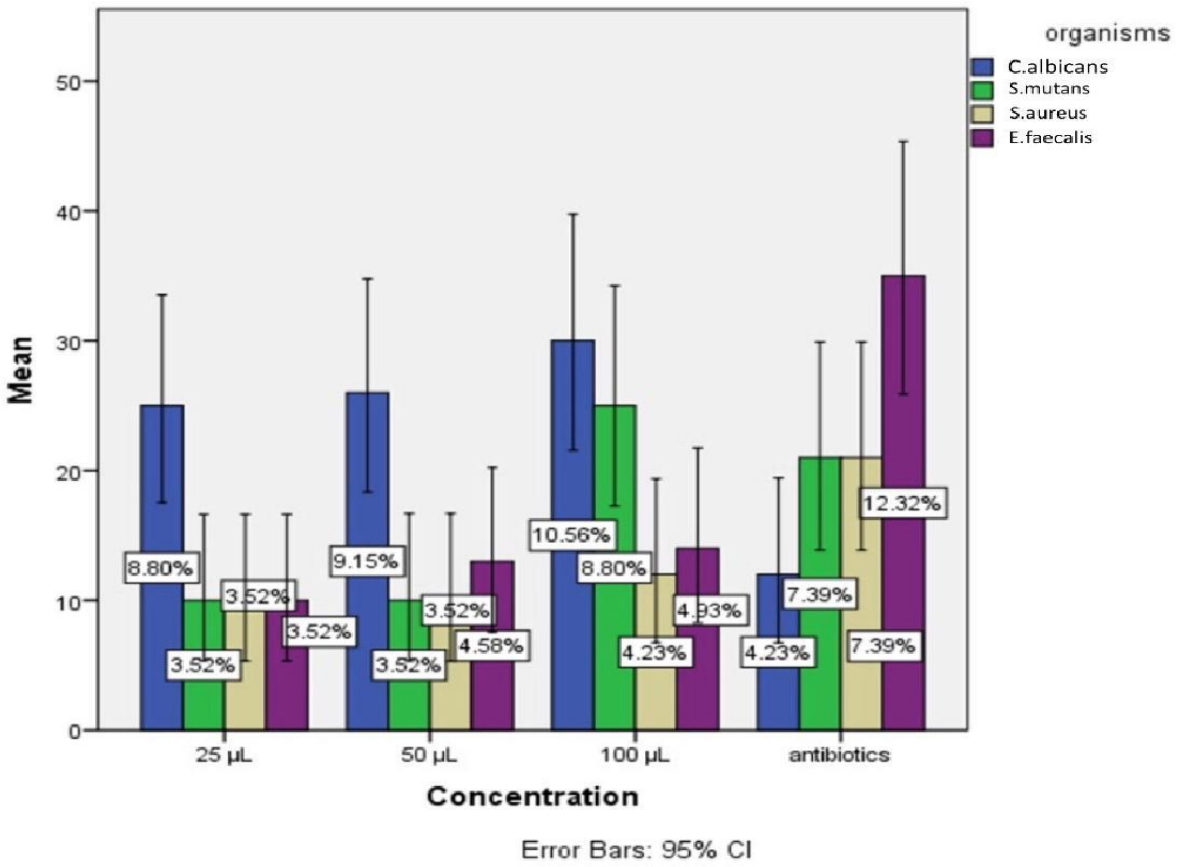

Fig. 7. Bar graph representing the anticariogenic activity of blue tea mediated copper nanoparticles at varying concentrations along with the positive control (amoxicillin)

The concentration was plotted on the X axis and the zone of inhibition was plotted on the Y axis. Here, blue represents the C.albicans, green represents the S.mutans , brown represents the S. aureus and violet represents the E. faecalis. At 25 µl and 100 µl, the anticariogenic activity against S. aureus was found to be statistically significant when compared to the standard ($p<0.05$). At 25 µl and 100µl the anticariogenic activity against C.albicans was found to be statistically significant when compared to the standard ($p<0.05$). At 25 µl, 50 µl and 100 µl, the anticariogenic activity against S.mutans was found to be statistically significant when compared to the standard ($p<0.05$). At 25 µl and 100 µl the anticariogenic activity against E. faecalis was found to be statistically significant when compared to the standard ($p<0.05$) (one way ANOVA followed by post hoc analysis).

Table 2. ANOVA test for anticariogenic activity

		Sum of squares	df	Mean square	F	Sig.
S. aureus	Between Groups	516.897	3	172.299	170.885	.000*
	Within Groups	8.066	8	1.008		
	Total	524.963	11			
C. albicans	Between Groups	521.056	3	173.685	170.928	.000*
	Within Groups	8.129	8	1.016		
	Total	529.185	11			
S. mutans	Between Groups	680.897	3	226.966	191.026	.000*
	Within Groups	9.505	8	1.188		
	Total	690.402	11			
E. faecalis	Between Groups	907.094	3	302.365	258.865	.000*
	Within Groups	9.344	8	1.168		
	Total	916.439	11			

**(p<0.05)*

Table 3. Post Hoc analysis for anticariogenic activity

Dependent variable	(I) Concentration	(J) Concentration	Sig.
S. aureus	25µL	50µL	.000*
	50µL	100µL	1.000
	100µL	25µL	.000*
	Antibiotic	25µL	.000*
		50µL	.999
		100µL	1.000
C. albicans	25µL	50µL	.000*
	50µL	100µL	.998
	100µL	25µL	.000*
	Antibiotic	25µL	.000*
		50µL	.021*
		100µL	.027*
S. mutans	25µL	50µL	.005*
	50µL	100µL	.000*
	100µL	25µL	.000*
	Antibiotic	25µL	000*
		50µL	.000*
		100µL	.080
E. faecalis	25µL	50µL	.000*
	50µL	100µL	.998
	100µL	25µL	.000*
	Antibiotic	25µL	.000*
		50µL	.000*
		100µL	.000*

**(p<0.05)*

4. DISCUSSION

The present study was done to assess the anticariogenic activity of copper nanoparticles synthesized using blue tea.

Blue pea blossom is phenomenal for the hair as well, as it contains anthocyanin - a compound known to build blood flow in the head and hence keep a sound scalp. It likewise helps in fortifying the hair follicles from the inside. Ready by soaking the lively indigo blossoms of the Clitoria ternatea plant in bubbling water, blue tea presents umpteen health motivations for a fit body and a sound psyche. Additionally prevalently known as butterfly pea blossom tea, this home grown invention is honored with a huge number of powerful cancer prevention agents like polyphenols, tannins, catechins, just as monstrous important phytonutrients, that show colossal memory helping, antihyperlipidemic, antihyperglycemic and pain relieving attributes. Beside granting a brilliant coloring, speeding up weight reduction, recharging skin tissues and enhancing hair development, some quite hot sans caffeine blue tea holds high helpful qualities in amending the side effects of heap diseases like diabetes, atherosclerosis, glaucoma and is really an empowering and supporting beverage. Enhances Diabetic Conditions, Tasting on some quite hot blue every day does something amazing in staying away from unexpected spikes in glucose levels, other than working with smooth assimilation processes, in those with type 2 diabetes mellitus. The plentiful stores of phenolic corrosive, phenolic amide cancer prevention agents in blue tea depict huge antihyperglycemic impacts, further developing insulin discharge, directing glucose digestion and forestalling abundance assimilation of sugars by body cells, both while fasting and post consuming suppers. Butterfly pea blossom tea is absolutely an optimal healthy expansion to the diabetic eating routine.

A study conducted by Balzani.et.al., stated that blue tea has a wide spectrum of medicinal applications due to its anti-inflammatory, antimicrobial, anticancer and wound healing properties [41,42]. Copper nanoparticles have effective antimicrobial action against a wide range of pathogens and also drug resistant bacteria [43]. Another study conducted by Hammad.et al., suggested that cinnamon and

clove exhibits excellent antimicrobial properties and plays a major role in herbal remedies and also exhibits anti inflammatory activity and antifungal properties [44].

A previous study by Kuo.et al., compared metal oxide nanoparticles and suggested that copper nanoparticles are comparatively inexpensive and relatively less toxic [45]. The anticariogenic activity of copper nanoparticles was assessed in liquid also as solid growth media [46], [47]. On solid media, the antibacterial characterization of the prepared NPs was measured by colony forming unit (CFU). In liquid media, the anticariogenic behaviour of copper nanoparticles was studied by determination of the optical density (OD). The results demonstrated that the anticariogenic efficacy of copper nanoparticles relied on the concentration of the nanoparticles; low concentrations just led to a delay within the lag phase, showing the micro nutritional role of copper for bacteria. In contrast, at higher concentrations, they showed anticariogenic growth inhibition [48].

The medicinal properties of blue tea have now drawn extensive attention. A study on *Clitoria ternatea* suggested that its methanolic extract could be a potential source of antioxidants and have a greater importance as therapeutically agents in preventing or slowing oxidative stress related degenerative diseases [49], [50], [51], 52]. This study assessed the anticariogenic activity of blue tea on potential oral pathogens [53] [54] [55] [56]. The study suggests that the blue tea clearly had an inhibitory effect on the growth of microbes such as *Staphylococcus aureus, Staphylococcus mutans, Enterococcus faecalis, Candida albicans.* However, these findings need to be confirmed with further clinical trials [57,58] [59] [60].

5. CONCLUSION

Within the limitations, the present study suggests that the blue tea mediated copper nanoparticles showed anticariogenic activity against *S. mutans, C. albicans, E. faecalis* and *S. aureus* and therefore can be used for clinical application.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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